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M. K. Semenova

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The role of copepods in the life cycle of *Contracaecum micropapillatum* (Ascaridata, Anisakidae)

by M.K. Semenova

At the present time, a great deal of attention is being devoted to the study of the host-parasite relations in the helminth-host system, as well as the role of various components of biocoenoses in the spread of the causative agents of helminthiases. Data concerning the study of helminths from ecological aspects are being used in the development of anthelminthic preventive measures.

In this paper, we discuss some of the aspects of the interrelations between the larval forms of the nematode *Contracaecum micropapillatum* and the constant components of aquatic biocoenoses, copepods (Copepoda). As we know, species of these crustaceans are frequently noted as the intermediate hosts of anisakids which are parasitic on animals ecologically related to aquatic biocoenoses. However, not all scientists agree on the role of copepods in the life cycles of nematodes of the genus *Contracaecum*. Some authors (Val'ter, 1968; Berland, 1961; Huisinga, 1966, etc.), being of the opinion that the nematodes of this genus develop with two intermediate hosts, believe that copepods play the role of the first intermediate host; other researchers (Punt, 1941, etc.) assume that nematodes of the genus *Contracaecum* develop with one intermediate host which may be associated with copepods. We are of the same opinion.

The discrepancy in the opinions regarding the intermediate hosts can be attributed to the fact that many of the aspects of the life cycles and ontogeny of nematodes from the genus *Contracaecum* have not been researched well enough, especially the morphology of the larvae at the initial stages of development, which does not enable us to explain some of the biological characteristics of these nematodes.

While studying the development of *C. micropapillatum*, a parasite of piscivorous birds, we attempted to establish the role of copepods in the life cycle of this helminth on the basis of a comparative morphological analysis of its larval forms. The experimental work was carried out in Daghestan on Agrakhan Gulf. The plankton for the experiments was collected from different habitats of the gulf (shallow waters, temporary bodies of water, scirpus growths, open deep-water areas), which are relatively rarely visited by piscivorous birds. In all the cases, 10-12% of the copepods designated for the experiments was examined for natural infestation by *Contracaecum* larvae. In addition to this, over 100,000 specimens of crustaceans from the suborder Cyclopoida and more than 2000 specimens from the suborder Calanoida caught near the rookeries and at the foraging grounds of piscivorous birds were subjected to compressor analysis to determine spontaneous infestation. Only in one of the crustaceans examined was a larva of the genus *Contracaecum* found. 500,000 crustaceans from the suborder Cyclopoida and 3000 from the suborder Calanoida were used in the experiments. Infection of the experimental copepods was carried out by free 2nd-instar larvae and larvae of the same instar still in the egg shell. The experimental crustaceans were examined daily, every 24 hours, for the first 20 days, and then with intervals of 5, 10, 20, 30, 40, 70, 100 and 120 days.

Results. During our experiments, we found that copepods act predominantly as an intermediate host in the life cycle of *C. micropapillatum*. Copepods are infested mainly by free-swimming 2nd-instar larvae, but they can also be infected by oscillating sessile larvae. The larvae are a food for copepods. The results of our experiments have shown that the members of the two suborders of copepods can serve as intermediate hosts for *C. micropapillatum*. It has been established that the intermediate hosts of this helminth include *Acanthocyclops vernalis*, *A. viridis*, *Cyclops strenuus*, *Macrocyclops albidus*, *M. fuscus*, *Mosocyclops crassus*, *M. leuckartii*, *Eucyclops macruroides*, *E. leuckartii*, *Arctodiaptomus salinus* and *Diaptomus gracilis*. In the majority of cases, these are representatives of the suborder Cyclopoida, and only two species (*Arctodiaptomus salinus* and *Diaptomus gracilis*) belong to the suborder Calanoida. The overall degree of infection of Cyclopoida amounts to 96-98% with the intensity of infection equal to 1-33

specimens. The degree of infection of *Diaptomus* is much lower, 6% and 1-2 specimens respectively. The experiments on infection of juvenile copepods have shown negative results.

The rate of larval development in copepods is greatly affected by the temperature factor. With the water at 22-25°C (optimal temperature under the conditions of our experiments), 2nd-instar larvae free themselves of the larval case in the intestine of the copepods within 25-30 minutes, and migrate to the body cavity. The main mass of larvae migrate within the first 30-40 minutes, but individual larvae can remain in the intestine of the copepods for up to 36-42 hours. Depending on their age (number of days after the first molt), the larvae undergo the second molt either shortly after invading the body cavity of the copepod, or after a certain period of growth inside the copepods. Our observations have shown that 2—3-day-old larvae remain in the copepods for 2-4 days, and then molt. In this case, the larvae usually molt on the 5-6th day after infection. 6—8-day-old larvae molt largely on the 2nd-3rd day after infection. The size of the molting larvae is the same in both cases; their length does not exceed 0.35-0.39 mm. Just before molting, the larvae become almost motionless, and the boundaries of the internal organs become barely discernible. The molting process lasts 24-36 hours.

Eight—ten hours following the molt of the 3rd instar, they become fairly active again. Because of their growth and increasing activity, the thin cuticular case surrounding the larvae ruptures, and is shed by the larvae 10-12 hours after completing the molt. After 24-36 hours, the larvae attain a length of 0.39-0.40 mm. After 15-20 days, 3rd-instar larvae double in length, and by the 30th day they become perceptibly less active. After that (at 73, 86 and 140 days), the 3rd-instar larvae do not show any significant increase in length, or any other morphological changes; only partial encapsulation of them is observed. 110-140 days after infection, all the larvae are enclosed in thin capsules, predominantly in the dorsal part of the body cavity of the copepods. When fed to fish and frogs (reservoir hosts), these larvae free themselves of their capsules in the intestine of the hosts, and migrate into the body cavity. Therefore, the ability of the larvae to infect

copepods is retained for quite a long time (140 days in our observations).

During our experiments, we found that 2nd-instar larvae that get into the body cavity of copepods can in some cases remain there without undergoing any morphological changes or molting for a fairly long period (26-41 days in our observations). During this time, the larvae grow only slightly in length, and some of them undergo partial encapsulation. These larvae grow to a length of 0.39-0.41 mm, which somewhat exceeds the length of larvae about to undergo the second molt. The motor activity of these larvae is very low. Passage of these larvae to frogs (*Rana ridibunda*) has shown that some of these larvae are capable of migrating and developing in new hosts. We regard these hosts as intermediate ones, and the copepods as reservoir hosts of 2nd-instar larvae. On the other hand, reservoir parasitism at the 2nd larval instar is not typical of this nematode, or of other representatives of the genus *Contracaecum*.

In our previous investigations (Semenova, 1973), we found that copepods (species of the genus *Macrocyclops*, *Cyclops*) could in some cases also consume small active 3rd-instar larvae which find themselves in the water after the intermediate copepod hosts die. In their new copepod hosts, the 3rd-instar larvae migrate, but they do not continue to grow or develop in these hosts. Consequently, in some cases, copepods can also act as a reservoir host for 3rd-instar larvae.

While conducting this particular study, we came across hosts of *C. micropapillatum* larvae, which, according to definitions in the literature, are neither intermediate, nor reservoir hosts. In experiments, we found these to be carnivorous planktonic copepods which feed actively on 2nd-instar larvae. In turn, the copepods infested by 2nd-instar larvae may be consumed shortly afterwards by other carnivorous invertebrates or fishes. With insufficient time to molt or even begin molting due to their brief presence in the copepods, the larvae can be taken over by new hosts in which they migrate and continue to develop. These new hosts, predominantly fish, take on the role of intermediate host, while the copepods are only a type of carrier or transport host; they transfer the 2nd-instar larvae from the environment into the intermediate hosts (other carnivorous invertebrates and fish). One can assume that this

occurs frequently in natural biocoenoses, and copepods, as carriers of larvae, play a significant role in the spreading of infection among invertebrates and fish.

Therefore, in the host-parasite system of *C. micropapillatum*, copepods can play the role of hosts of different categories, i.e. intermediate, reservoir host of 2nd-instar larvae, reservoir host of 3rd-instar larvae, and transport host of 2nd-instar larvae. However, copepods are primarily necessary as stadiogenic hosts in the ontogeny of *C. micropapillatum* and probably other species of the genus *Contracaecum*. In certain bodies of water, copepods can represent an important link in the chain of transmission of contracaecoses from invertebrates to fishes. The species of copepods studied by us probably do not play any significant role in the elimination of *C. micropapillatum* larvae. Since copepods can be hosts for several categories of nematodes of the genus *Contracaecum*, the density of these crustaceans should be taken into account when evaluating the waters of different climatic and topographic zones with regard to diseases caused by nematodes of the genus *Contracaecum*.

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